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Dietary nano-silver: Does support or discourage thermal tolerance and biochemical status in air-breathing fish reared under multiple stressors?

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ABSTRACT

Unexpected fluctuations in weather parameters due to global climate change have been observed in all ecosystems worldwide. The aquatic ecosystem shelters a great diversity of fishes in the upper region of the ecosystem which adversely get affected due to their poikilothermic nature. The present study was designed to elucidate the impact of critical temperature minima (CTMin), lethal temperature minima (LTMin), critical temperature maxima (CTMax), and lethal temperature maxima (LTMax) on *Channa striatus*. Biologically synthesized silver nanoparticles (Ag-NPs) were evaluated for their potential to enhance thermal tolerance and improve the activities of biochemical enzymes of *C. striatus* reared under lead (Pb) and high temperature (34 °C) for 50 days. Three iso-caloric and iso-nitrogenous diets which included a basal diet and two supplemented diets with Ag-NPs @ 0.5 mg/kg, and 1 mg/kg were used in the study. Results suggested that CTMin and LTMin were significantly ($p < 0.01$) reduced and CTMax and LTMax were enhanced in the group fed with 0.5 mg/kg Ag-NPs supplemented feed. Pre-exposure to high temperature led to enhanced CTMax and LTMax in *C. striatus*. The biochemical enzymes involved in protein metabolism, carbohydrate metabolism, acetylcholine esterase and antioxidant activities were found to be normal in fish fed with 0.5 mg/kg Ag-NPs supplemented diet. Bioaccumulation of silver and Pb was determined in different fish tissues and experimental water. Overall, the incorporation of Ag-NPs at 0.5 mg/kg in diet can confer protection to fish against Pb and thermal stress and enhance thermal tolerance of *C. striatus*.

1. Introduction

The world has witnessed enormous changes in low and high temperatures due to as a result of global climate change. The sudden change of climate observed throughout the year irrespective of the season in all the continents, is a serious concern with respect to aquatic animals. The global warming is a strong precursor of vulnerability to heavy metals, pesticide residue and household chemicals in fish, wildlife and, also humans (Lovett, 2009). The complex interactions between chemicals due to the climate change might be making chemicals more toxic and the environment more susceptible to damage (Lovett, 2009). The global climate change affects all the living organism including fish, which are more vulnerable due to their ectothermic nature. Fish growth development, ontogenesis and metabolism also depend on the thermal regime and change in the ambient temperature lead to a shift in the metabolic regulation system (Goncealves et al., 2010; Deutsch et al., 2015). Generally, temperature fluctuations go beyond optimal conditions in case of thermal stress condition (Axenov-Gribanov et al., 2016). The biochemical pathway attributes are major indicators of the ability of ectotherms to acclimate to a new stable state and are recognised as the key processes by which organisms will, or will not cope with climate warming (Portner, 2002a; Calosi et al., 2008; Peck et al., 2010; Morley. et al., 2012). The thermal tolerance of animals including fish involve s the physiological and biochemical mechanisms responsible for thermal acclimation and any alterations in these may interfere with biochemical pathways in which can affect whole animal performance (Morley. et al., 2012). Under such condition, the limited capacity to provide oxygen to tissue and cover temperature-dependent oxygen demand initiates thermal limitation (Tripp-Valdeza et al., 2017). When temperature crosses the optimal range, the cardio-ventilatory capacity

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of the organism fails to meet the rising demand as indicated by the development of systemic hypoxemia of the fish (Frederich and Portner, 2000). By temperature manipulation experiments, studies have shown that metabolic rate and life-span have a direct correlation to each other (Kumar et al., 2014a, 2016a; 2017a, 2017b, 2017c; Tejpal et al., 2014).

It is a great concern for scientists that how aquatic animals can adopt, mitigate and survive in global climate changes and perform well similar to a natural resident in spite of stressors condition. Nutritional approaches may be the best option to sustain aquatic animals in climate change along with concurrent exposure to heavy metal and elevated temperature. Our earlier study revealed that through dietary manipulation, the immune response of fish can be improved and at the same time, thermal tolerance against multiple stressors such as Pb and high temperature (Kumar et al., 2017a, 2017b, 2017c; Tejpal et al., 2014) can be enhanced. The use of dietary mineral nanoparticles has been used for the aquatic animal as a feed ingredient for efficient use in the fish feed (Kumar et al., 2017a, 2017b, 2018a; Muralisankar et al., 2014; Zhao et al., 2014). Silver nanoparticles are used as an antimicrobial agent (Elechiguerra et al., 2005; Shahverdi et al., 2007), growth promoter (Sarkar et al., 2015), immune booster (Beck et al., 2015), in treating wounds and burns (Samuel and Guggenbichler, 2004) in animal and human. The study on role of Ag-NPs on immunity and redox response in animals are very scanty (Pineda et al., 2012; Bhanja et al., 2015; Kumar et al., 2018a). Ag-NPs are carriers of available oxygen (O_2) that fit in the octahedral holes of Ag-NPs accumulated in the tissues. However, it may increase anabolic activity and subsequently stimulate growth and immunity (Pineda et al., 2012). Hence, we evaluated dietary Ag-NPs for enhancement of thermal tolerance and improvement of biochemical attributes in *C. striatus*. Air-breathing fish *Channa striatus*, commonly known as snake head murrel or striped snake head, is known for hypoxia tolerance. It is a commercially important fish used for therapeutic purpose, enhancing wound healing and reducing postoperative pain (Mollah et al., 2009). The physiological tolerance against depleted oxygen concentration in *C. striatus* is due to the presence of special air-breathing organ respiring through the air (Gamperl et al., 2004; Overgaard et al., 2004).

During lethal temperature and exposure to Pb, the production of free radical is enhanced resulting in alteration of antioxidative enzymes, neurotransmitter enzyme, protein and carbohydrate metabolic enzymes (Kumar et al., 2017c). Due to more demand of oxygen to the cell, exacerbated mismatch between oxygen supply and demand finally leads to the onset of anaerobic metabolism (Portner, 2010). The impact of Pb and high temperature alone or in concurrence affects the thermal tolerance of the fish. In this context, the present study was carried out with the aim to evaluate the possible protective role of silver nanoparticles (Ag-NPs) against thermal stress reared under lead (Pb) and high temperature (34 °C) of *C. striatus*.

2. Materials and methods

2.1. Ethics statement

The use of animals conforms to the existing laws in India. The care and treatment of animals used in this study were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals, [Ministry of Environment & Forests, Animal Welfare Division, Government of India]. The study protocol and experimental endpoints were approved by the institute research committee and the authorities of the ICAR-National Institute of Abiotic Stress Management, Baramati, Pune, India.

2.2. Experimental animal, design and condition

The experimental animal (Snake head murrel, *Channa striatus*) was collected from Nira canal, Malegaon, Pune district in Maharashtra, India. Murrel was quarantine with salt solution (2%) and acclimated in fibreglass reinforced plastic (FRP) tanks (Circular, 500 L) for twenty days prior to the experiment. For the thermal tolerance study, *Channa striatus* was randomly distributed into 18 glass aquaria (60 × 47 × 32 cm) of 60 L capacity and reared for 50 days. Fifteen fish of uniform size (weight, 3.92 ± 0.75 g) per tank was stocked in six distinct treatment groups in triplicate following a completely randomized design. Fish were fed with the experimental diet twice daily (10:00 am and 17:00 pm) to satiation throughout the experimental period. Round-the-clock aeration was provided to all the tanks from a compressed air pump and manual water exchange (two third) was carried out at every second day. The experimental setup consisted of normal water and fed with the control diet (control group, Ctr/Ctr), concurrent exposed to Pb and temperature (34 °C) and fed with control diet (LT), normal water and fed with Ag-NPs @ 0.5 mg/kg (Ag-NPs @ 0.5 mg/kg), normal water and fed Ag-NPs @ 1 mg/kg (Ag-NPs @ 1 mg), concurrent exposed to Pb and temperature exposed (34 °C) and fed with Ag-NPs @ 0.5 mg/kg (Ag-NPs @ 0.5 mg-LT), Pb treated and temperature exposed (34 °C) and fed with Ag-NPs @ 1 mg/kg (Ag-NPs @ 1 mg/diet-LT). The Pb was maintained at the level of 1/25 (4 ppm) of 96 h LC_{50} for all the treatments using lead nitrate (Thermo Fisher Scientific India Pvt. Ltd. Mumbai, India). The temperature was measured with the help of probe based aquarium thermometer (normal 26.4–28.8 °C and 34 ± 0.7 °C in temperature treated groups). Water quality parameters such as dissolve oxygen (6.4 ± 0.45), pH (7.43 ± 1.23), free carbon dioxide (ND), total hardness (234.62 ± 3.86 mg/L), ammonia (0.14 ± 0.02 mg/L), nitrite (0.002 ± 0.001 mg/L) and nitrate (0.04 ± 0.02 mg/L) were recorded as per APHA (1998).

2.3. Experimental diet

Three iso-calorific and iso-nitrogenous diets (35% crude protein) viz. basal diet (Ag-NPs free control diet) and two supplemented diets viz., 0.5 mg/kg, and 1 mg/kg of Ag-NPs were prepared. Silver nitrate (HI-MEDIA, Mumbai, India) was used for the synthesis of silver nanoparticles and mixed in the experimental feed @ 0.5 mg and 1 mg/kg diet. For the formulation of pelleted diet, quality fish meal, soybean meal, sunflower meal, wheat flour, sunflower oil and cod liver oil were procured from local market. Silver free vitamin mineral mixtures were prepared manually along with ascorbyl phosphate (SD Fine Ltd., Mumbai, India) used as the source of vitamin C. The dough was mixed properly, pelleted, air dried and kept in the hot air oven at 60 °C until dried (Table 1).

Table 1
Ingredients composition of the different experimental diets fed to *Channa striatus* during the experimental period of 50 days.

	Control	Nano-silver (0.5 mg)	Nano-silver (1 mg)
Soybean meal[a]	35.5	35.5	35.5
Fish meala	20	20	20
Sunflower meal[a]	10	10	10
Wheat flour[a]	26.470	26.4695	26.4690
Sunflower oil[a]	4.5	4.5	4.5
Cod liver oil[a]	1.5	1.5	1.5
Vitamin + mineral mixb*	2	2	2
Vitamin Cc	0.03	0.03	0.03
Nano-silver	0	0.0005	0.001
	100	100	100

Composition of vitamin mineral mix (quantity/250 g starch powder): vitamin A 55,00,00 IU; vitamin D3 11,00,00 IU; vitamin B1:20 mg, vitamin B2 2,00 mg; vitamin E 75 mg; vitamin K 1,00 mg; vitamin B12 0.6 mcg; calcium pantothenate 2,50 mg; nicotinamide 1000 mg; pyridoxine: 100 mg; Mn 2700 mg; I 1,00 mg; Fe 750 mg; Cu 200 mg; Co 45 mg; Ca 50 g; P 30 g; selenium 0.5 ppm.

[a] Procured from local market.

[b] Prepared manually and all components from Himedia Ltd.

[c] SD Fine Chemicals Ltd., India.

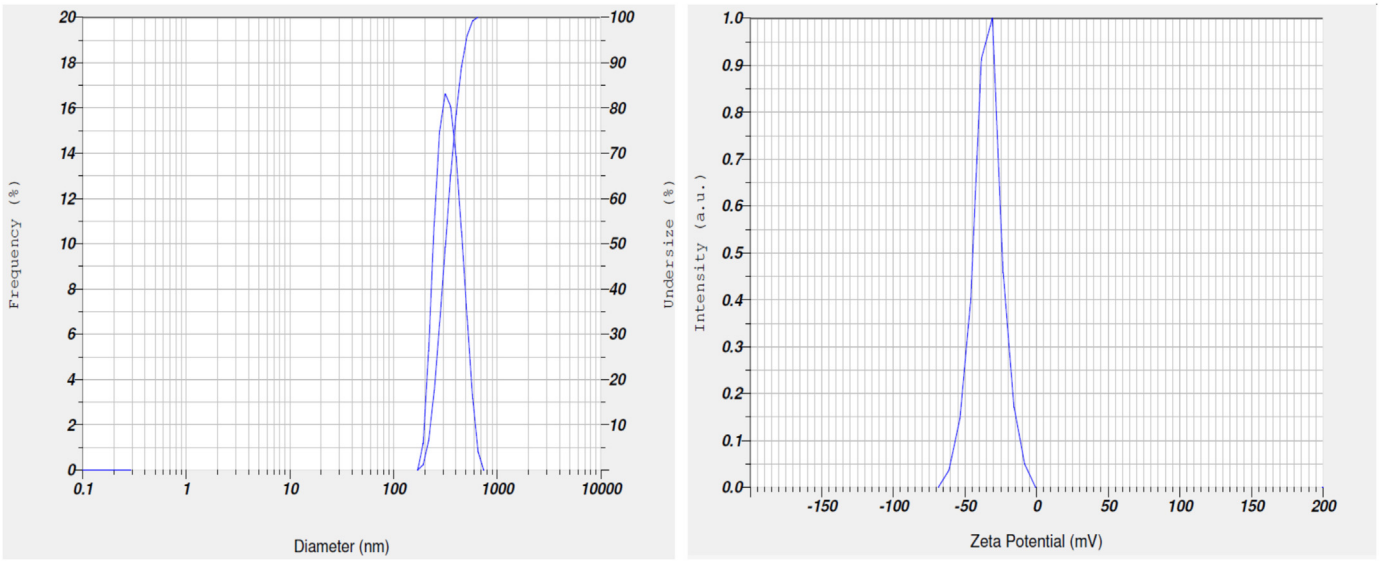

Fig. 1. Size (297 nm) and zeta potential (-34 mV) of silver-nano particles.

2.4. Green synthesis of silver nanoparticles (Ag-NPs)

2.4.1. Preparation of fish tissue extract

Green synthesis of Ag-NPs from fish waste was prepared from the complete gill of live *Channa striatus*. The dissected tissues were washed and chopped into small pieces. The homogenates were centrifuged at 5000–6000 rpm, then the supernatant was collected, and then filtrate was mixed with 200 ml of aqueous solution of silver (200 mM) using the previous protocol (Sinha et al., 2014, 2015; Kumar et al., 2018c).

2.4.2. Characterization of silver nanoparticles

The synthesized Ag-NPs was evaluated through an absorption spectrum at 300–500 nm in UV–Vis spectrophotometer and the observed peak obtained in the range of 360–380 nm. The synthesized Ag-NPs formulations were diluted with Milli-Q water and processed for particle size analysis through a size analyzer. The mean size of Ag-NPs was found to 297 nm and mean zeta potential −34 mV (Horiba Scientific Nanoparticles Analyzer nano Partica SZ-100 series, Kyoto, Japan) at 25 °C (Fig. 1).

2.5. Thermal tolerance experiment

Thermal tolerance was determined as described by Lutterschmidt and Victor (1997), Beitinger et al. (2000), Kumar et al. (2014a; 2016a; 2017a, 2017b, 2017c) and Dalvi et al. (2009). The fish were deprived of feed for one day before performing the thermal tolerance study. Four fish (two for CTmin & LTmin and two for CTmax & LTmax) were randomly selected from each replicate from each treatment group. Fish from each treatment group were shifted to separate thermostatic water bath aquaria (Thermo Scientific Instruments & Equipment's, Waltham, MA, USA, sensitivity ± 0.2 °C) for the temperature tolerance study. The temperature and lead concentration of water in the thermostatic aquaria were maintained similar to the experimental groups. Dissolved oxygen was maintained at 6.5 ± 0.5 mg/L throughout the temperature tolerance study by continuous aeration using a 2-HP centralized air blower. Water temperature in the aquarium was increased /decreased at a constant rate of 0.30 °C/min, until the loss of equilibrium (LOE) is reached, which was designated as the CTmax / CTmin (Dalvi et al., 2009; Kumar et al., 2014a; 2016a; 2017a, 2017b, 2017c). The lethal thermal maximum (LTmax) / lethal thermal minimum (LTmin) were determined by further increasing /decreasing the temperature until the opercular movement was ceased (Tsuchida, 1995; Dulger et al., 2012). The LOE, indicates a physiological condition where the brain fails to maintain balance, but somehow try to survive by taking oxygen through opercula whereas loss of opercular movement is a condition where fish fails to move the opercula and ultimately die. Das et al. (2004) and Kumar et al. (2014a; 2016a) have critically evaluated this technique and it is well established as a powerful tool for studying the thermal tolerance in fish (Paladino et al., 1980; Baker and Heidinger, 1996).

2.6. Sample preparation for different biochemical parameters

Fish were anesthetized with clove oil at 50 µl L^{-1} and subsequently dissected under aseptic conditions to collect tissues such as gill, liver, brain, kidney and muscle then homogenized (5% w/v) separately in chilled sucrose solution (0.25 M) in a glass tube using Teflon coated mechanical tissue homogenizer (Omni Tissue Master Homogenize, Kennesaw, GA). The tubes were kept on ice to avoid denaturation of the enzymes during the homogenization. The homogenates were centrifuged at 5000 rpm for 20 min at 4 °C in a cooling centrifuge (Eppendorf AG, 5430 R, Hamburg, Germany). Protein contents in the supernatants were quantified following the method of Lowry et al. (1951) using bovine serum albumin as a standard. The supernatants were collected and stored at −20 °C until further analysis.

2.7. Antioxidant enzyme activities

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured by the method of Misra and Fridovich (1972). The assay was based on the oxidation of epinephrine-adrenochrome transition by the enzyme. Catalase (CAT) (EC 1.11.1.6) activity was determined by the method of Takahara et al. (1960). The solution of 2.45 ml phosphate buffer (50 mM; pH-7), 50 µl tissue homogenate and 1 ml of hydrogen peroxide substrate solution (freshly prepared) were mixed well and decrease in absorbance was read at 240 nm for 3 min. Gluthathione-S-transferase (GST) (EC 2.5.1.18) was measured by the method of Habing et al. (1974). The S-2, 4-dinitrophenyl glutathione (CDNB) was used as a substrate. The method was based on the principle of formation of an adduct of CDNB, S-2, 4-dinitrophenyl glutathione.

2.8. Metabolic enzymes

Aspartate aminotransaminase (AST; EC.2.6.1.1) and alanine amino transaminase (ALT; EC.2.6.1.2) activities were measured by the estimation of oxaloacetate and pyruvate released, respectively, after incubating the reaction mixture at 37 °C for 60 min (Wootton, 1964).

Lactate dehydrogenase (LDH; L-lactate NAD1 oxidoreductase; EC.1.1.1.27) was assayed using 0.1 M phosphate buffer (pH 7.5) and 0.2 mM NADH solution in 0.1 M phosphate buffer. The reaction was initiated with the addition of substrate 0.2 mM sodium pyruvate and absorbance was recorded at 340 nm (Wroblewski and LaDue, 1955). A similar reaction mixture was used for the estimation of malate dehydrogenase (MDH; L-malate: NAD + oxidoreductase: EC.1.1.1.37) except the substrate (1 mg oxaloacetate/ml of chilled triple distilled water) and absorbance was recorded at 340 nm (Ochoa, 1955).

2.9. Neurotransmitter enzyme activities

Acetyl cholinesterase (AChE; EC 3.1.1.7) was measured by the method of Hestrin (1949). The activity was spectrophotometrically measured as the increase in the absorbance of the sample at 540 nm.

2.10. Sample preparation for silver and lead (Pb) analysis in water, feed and muscle tissue

Muscle tissue of 0.5 g was collected during dissection for analysis of Ag-NPs and Pb followed by acidic digestion in microwave digestion system (Microwave Digestion System, Model START-D, SN- 135177, Milestone, USA). HNO_3 and H_2O_2 were added in 5:1 ratio for digestion and then completely digested samples were allowed to cool to room temperature, then, digested samples were filtered with Whatman paper with 0.45 μm pore size. The final volume made up to 50 ml to proceed further for Ag-NPs and Pb analysis through inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 series, Agilent Technologies, USA). Silver concentrations in feed and fish tissues and Pb concentrations in water and fish tissues were determined through ICP-MS. Multi-element calibration standard (Agilent Technologies, USA) solutions of 10 μg/ml were used to prepare for the calibration curve. The calibration curves with $R^2 > 0.999$ were accepted for concentration calculation (Kumar et al., 2017d, 2017e, 2017f).

2.11. Experimental setup

Treatment 1: (control group, Ctr/Ctr), Normal water and fed with the control diet.

Treatment 2: (LT), Concurrent exposure to Pb and temperature (34 °C) and fed with control diet.

Treatment 3: (Ag-NPs @ 0.5 mg/kg), Normal water and fed with Ag-NPs @ 0.5 mg/kg. Treatment 4: (Ag-NPs @ 1 mg), Normal water and fed Ag-NPs @ 1 mg/kg.

Treatment 5: (Ag-NPs @ 0.5 mg-LT), Concurrent exposure to Pb and temperature (34 °C) and fed with Ag-NPs @ 0.5 mg/kg.

Treatment 6: (Ag-NPs @ 1 mg/diet-LT), Pb treated and temperature exposed (34 °C) and fed with Ag-NPs @ 1 mg/kg.

2.12. Statistical analysis

The data were statistically analysed by the statistical package for the social sciences (SPSS) version 16.0 (SPSS, Chicago, IL), in which data were subjected to one way ANOVA followed by Duncan's multiple range tests to determine the significant differences between the means. Comparisons were made at 1% and 5% probability level.

3. Results

3.1. Thermal tolerance of Murrel (Channa striatus)

The impact of silver nanoparticles (Ag-NPs) for improvement of thermal tolerance of *C. striatus* has been illustrated in Fig. 2. The dietary supplementation of Ag-NPs @ 0.5 mg/kg diet has significantly improved ($p < 0.01$) the thermal tolerance (CTMin, LTMin, CTMin and CTMax) of *C. striatus* concurrent exposed to Pb and high temperature in different experimental groups. CTmin and LTmin was remarkably reduced ($p < 0.01$) with supplementation of Ag-NPs @ 0.5 mg/diet with or without stressors (Pb and temperature) and control group in comparison to exposure group of Pb and high temperature and fed with control diet and Ag-NPs @ 1 mg/kg diet with or without stressors group. The lowest CTmin was observed in the group fed with supplementation of dietary Ag-NPs @ 0.5 mg/kg diet with or without stressed group (15.81 ± 0.63) and control fed group (15.17 ± 0.56). Similarly, lowest LTmin were noticed in the group fed with control diet (14.57 ± 0.33) and Ag-NPs diet without stressors (15.27 ± 0.32) followed by Ag-NPs @ 0.5 mg/diet with stressors (15.86 ± 0.29) group. Moreover, highest CTmax were observed in the group fed with control and concurrently exposed to Pb and temperature (46.26 ± 0.98) and Ag-NPs @ 0.5 mg/kg diet exposed to Pb and high temperature (45.46 ± 0.66) followed by control (43.74 ± 0.53) and Ag-NPs fed group (43.55 ± 0.63). Similarly, highest LTMax were noticed in the group exposed to Pb and temperature and fed control diet (47.14 ± 0.70) and Ag-NPs @ 0.5 mg/kg diet (46.22 ± 0.52). The positive correlation was also observed between CTMin, LTmin (0.76 + 1.01 X, R^2, 0.995) and CTMax, LTmax (0.27 + 1.0 X, R^2, 0.992) treated with Pb and high temperature and fed with Ag-NPs diet.

3.2. Oxidative stress during LTmin and LTMax of C. striatus

Secondary stress response in term of CAT, SOD and GST were evaluated in liver, gill, brain and kidney of *C. striatus* during LTmin and LTmax and illustrated in Figs. 3–5. CAT activities in liver, gill, brain and kidney of *C. striatus* were significantly ($p < 0.01$) higher in group exposed to Pb and high temperature and fed with control diet and Ag-NPs @ 1 mg/kg diet compared to supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors group and control diet group during LTMin and LTMax (Fig. 3). CAT activities were noticeably reduced ($p < 0.01$) with supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors group during LTMin and LTMax. The supplementation of Ag-NPs @ 1 mg/kg diet with or without stressors group showed elevated levels of CAT activities in comparison to Ag-NPs @ 0.5 mg/kg diet and control diet group. The supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors (Pb and high temperature) had noticeably ($p < 0.01$) reduced CAT activities in liver, gill, brain and kidney tissues of *C. striatus* during LTmin and LTmax. Similarly, SOD activities (Fig. 4) in liver, gill, brain and kidney were highly elevated ($p < 0.01$) in group exposed to Pb and high temperature and fed with control diet and supplementation of Ag-NPs @ 1 mg/kg diet with or without stressors during LTMin and LTMax. The supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors and control fed group significantly ($p < 0.01$) reduced SOD activities in all tissues in compared to all other groups during LTMin and LTMax. Moreover, activities of GST in liver, gill, brain and kidney were also significantly reduced by supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors and group fed with control diet compared to all other groups during LTMin and LTMax (Fig. 5).

3.3. Metabolic stress response

Metabolic stress response in terms of protein metabolic enzymes (ALT and AST) and carbohydrate metabolic enzymes (LDH and MDH) of *C. striatus* are illustrated in Figs. 6, 7. AST and ALT activities in liver and gill tissue were remarkably reduced ($p < 0.01$) with supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors and control fed group in comparison to all other groups. The highest AST and ALT activities in liver and gill were observed in the group concurrently exposed to Pb and temperature and fed with control diet as well as Ag-NPs @ 1 mg/kg diet. In case of gill, AST activities were found to be lowered in group fed with Ag-NPs @ 0.5 mg/diet followed by control group and Ag-NPs @ 0.5 mg/diet reared under stressors group and highest activities were observed in the group fed with Ag-NPs @

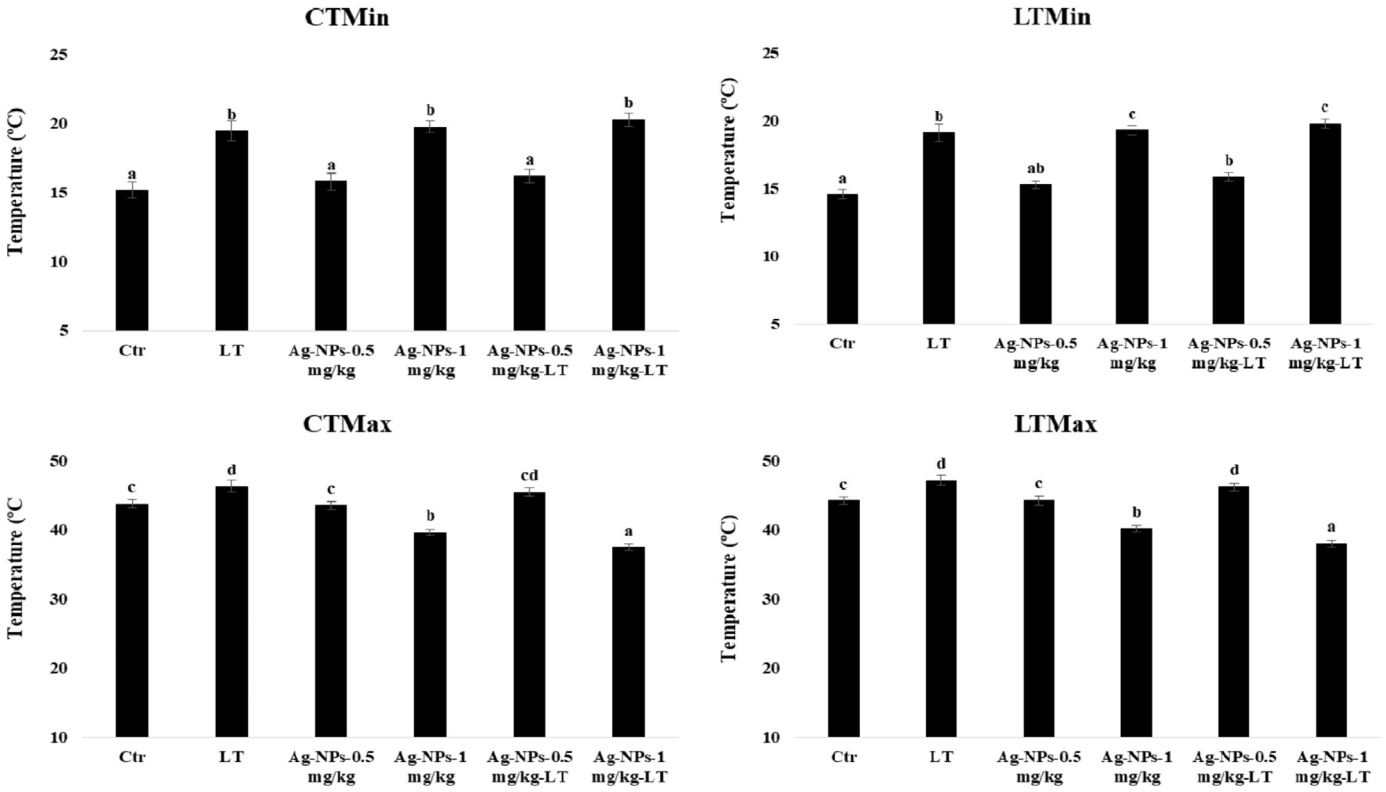

Fig. 2. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on critical thermal minima (CTMin), lethal thermal minima (LTMin) and critical thermal maxima (CTMax), lethal thermal maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d) indicate significant differences.

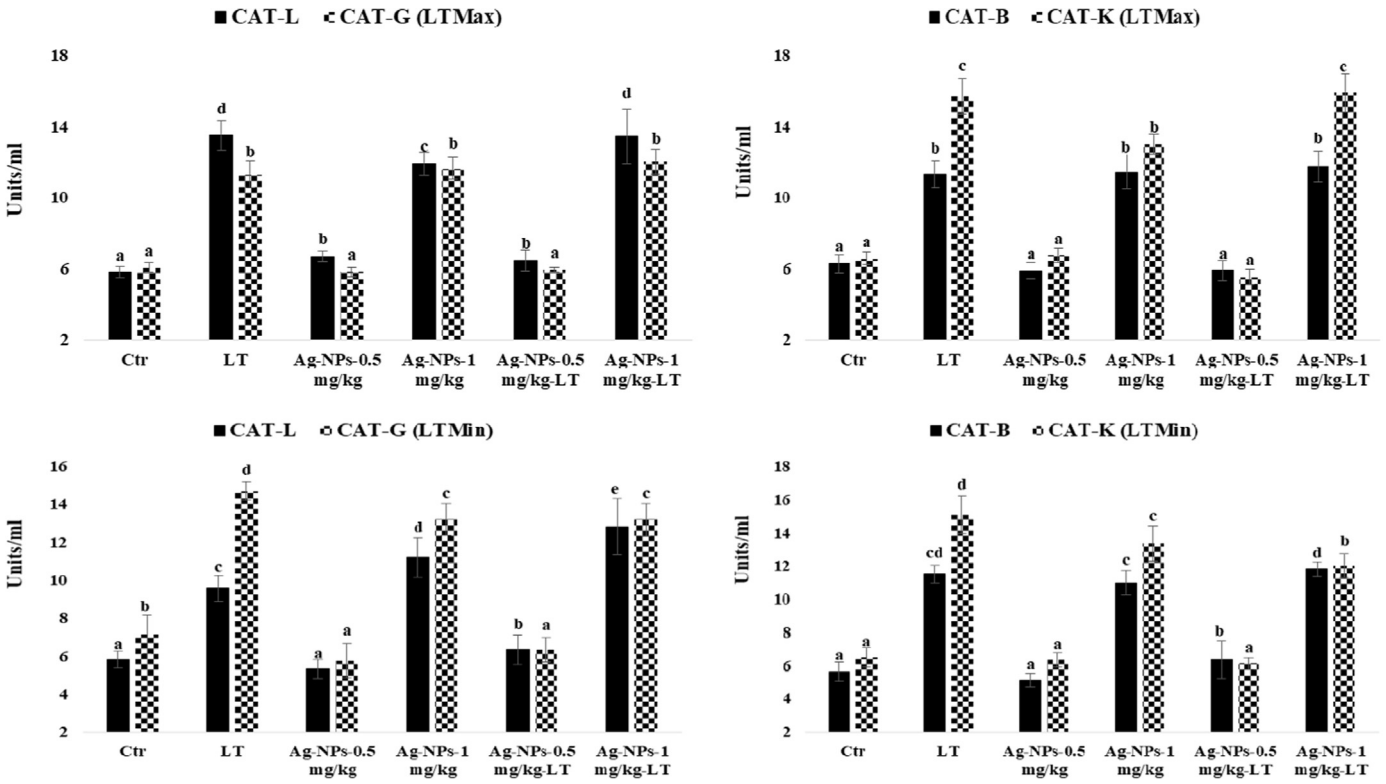

Fig. 3. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on catalase (CAT) activities in liver, gill, brain and kidney after lethal temperature minima (LTMin) and lethal temperature maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d) indicate significant differences.

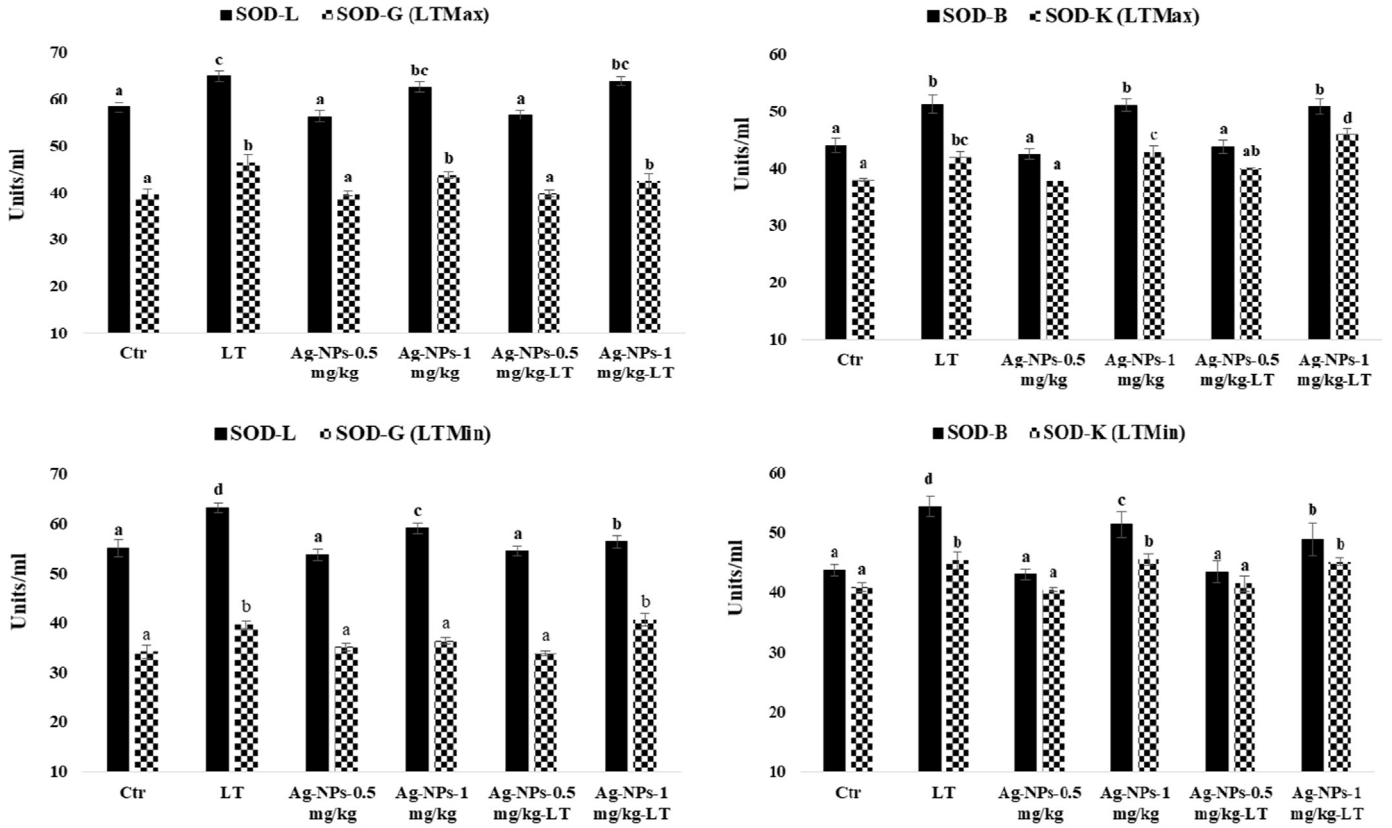

Fig. 4. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on superoxide dismutase (SOD) activities in liver, gill, brain and kidney after lethal temperature minima (LTMin) and lethal temperature maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d, e) indicate significant differences.

1 mg/kg diet under stressors during LTMin (Fig. 6). LDH and MDH activities in muscle tissue were significantly ($p < 0.01$) lowered in group fed with Ag-NPs @ 0.5 mg/kg diet and control diet fed group followed by Ag-NPs @ 0.5 mg/kg diet, exposed to Pb and high temperature during LTmin and LTMax. The highest activities of LDH and MDH were observed in the group treated with Pb and high temperature and fed with control diet and Ag-NPs @ 1 mg/kg diet (Fig. 7).

3.4. Neurotransmitter enzymes

The neurotransmitter enzymes in term of acetylcholine esterase (AChE) of *C. striatus* is summarised in Fig. 7. Dietary supplementation of Ag-NPs @ 0.5 mg/kg diet found to have significant elevation ($p < 0.01$) in the AChE activities in brain tissue of stressed and non-stressed fish. The activities were drastically reduced ($p < 0.01$) with the group treated with Pb and high temperature and fed with control diet as well as Ag-NPs @ 1 mg/kg diet during LTMin and LTMax.

3.5. Silver (Ag) and lead (Pb) analysis in fish tissues and experimental water

The concentration of Ag and Pb was analysed in muscle, liver, gill, kidney and experimental water at the end of the experimental (Table 2) period. The concentration of Ag in muscle, liver, gill and kidney tissues varied from 0.02 to 0.08, 0.05 to 0.18, 0.01 to 0.05, 0.04 to 0.19 mg/kg, respectively. Similarly, the Ag concentration in experimental water varied from 0.14 to 0.33 µg/L. Moreover, the Pb concentration varied from 0.01 to 0.45, 0.02 to 0.62, 0.01 to 0.33 and 0.04 to 0.95 mg/kg in muscle, liver, gill and kidney tissues, respectively. Pb concentration in experimental water varied from 0.17 to 602.23 µg/L.

4. Discussion

The thermal tolerance of the fish depends on several factors such as water quality, species, age, migration, feeding habit, and quality of feed ingredients. In the present study, we designed an experiment employing dietary Ag-NPs for enhancement of thermal tolerance of *C. striatus* reared under Pb and high temperature. Silver nanoparticles were synthesized by adopting green synthesis process. The relation between Ag-NPs and temperature (thermal tolerance) has not been reported earlier. Hence, the present study is the first report on the role of Ag-NPs for enhancement of thermal tolerance in *C. striatus*.

The aquatic bodies get heated due to the climate change resulting in alteration of fundamental ecological processes and the geographic distributions of aquatic species (Poff et al., 2002). The climate change is likely to be a stress creator for freshwater and coastal wetlands which are already adversely affected by varieties of other human impacts such as altered flow regimes and deterioration of water quality (Kevin, 2009). In our present study, the highest CTMax and LTMax have been observed in the pre-exposure of temperature and Pb. The group supplemented with Ag-NPs @ 0.5 mg/kg diet exposed to Pb and temperature showed similar CTMax and LTMax. However, it is inferred from the present results that pre-exposure to temperature enhanced the capacity of thermal tolerance in fishes as also indicated from enhanced CTMax and LTMax (upper thermal tolerance), which corroborated well with other reports (Das et al., 2004; Debnath et al., 2006; Dalvi et al., 2009; Kumar et al., 2014a; 2017a, 2017b, 2017c; 2016b). Generally, the temperature is the key factor for fish which are poikilothermic animals. However, if the temperature rises or falls beyond optimum levels, the haemostasis in fish gets disturbed resulting in a reduction of growth performance, thermal tolerance, metabolic performance as well as the reproductive success of the fish (Fry, 1971).

In the present study, the group supplemented with Ag-NPs @

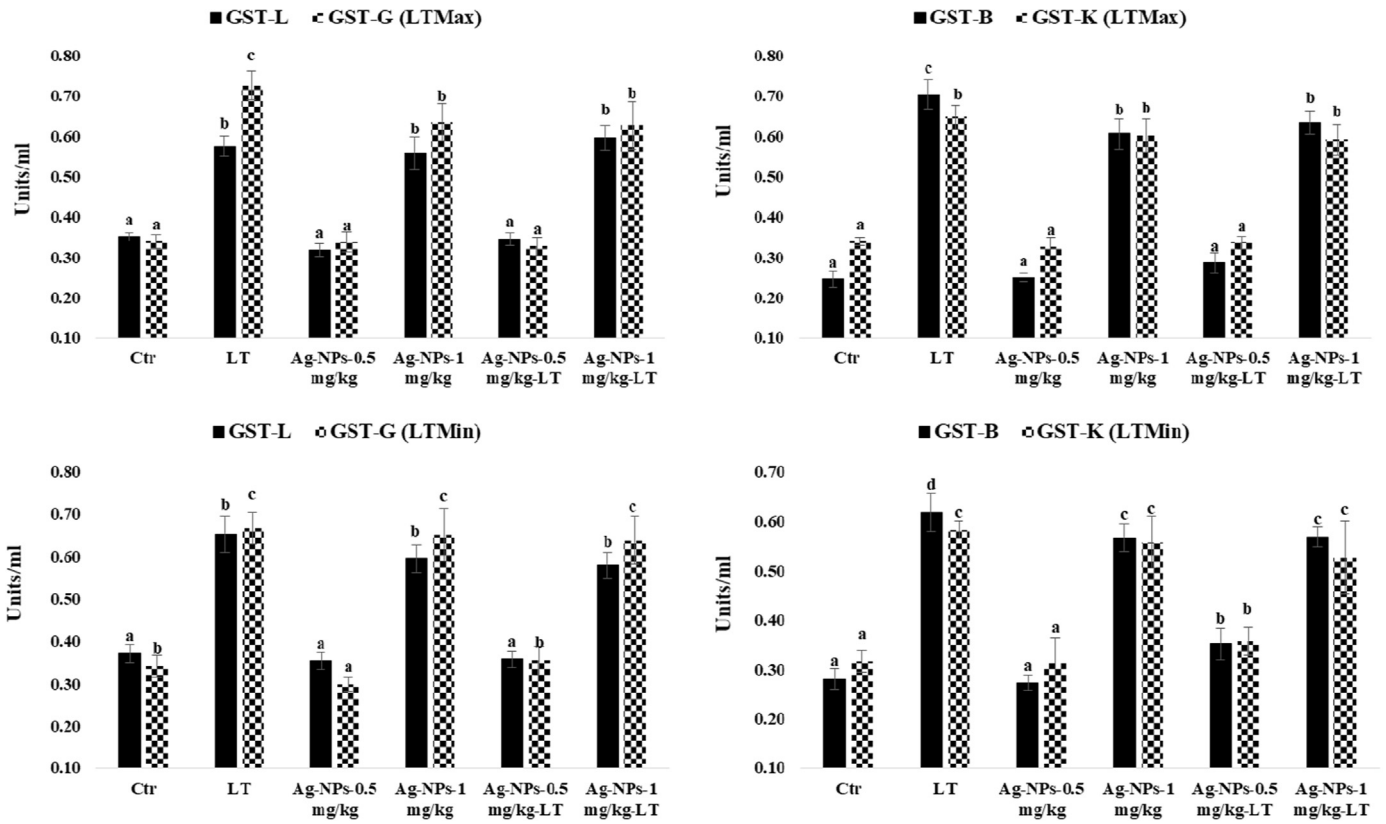

Fig. 5. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on glutathione-s-transferase (GST) activities in liver, gill, brain and kidney after lethal temperature minima (LTMin) and lethal temperature maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d) indicate significant differences.

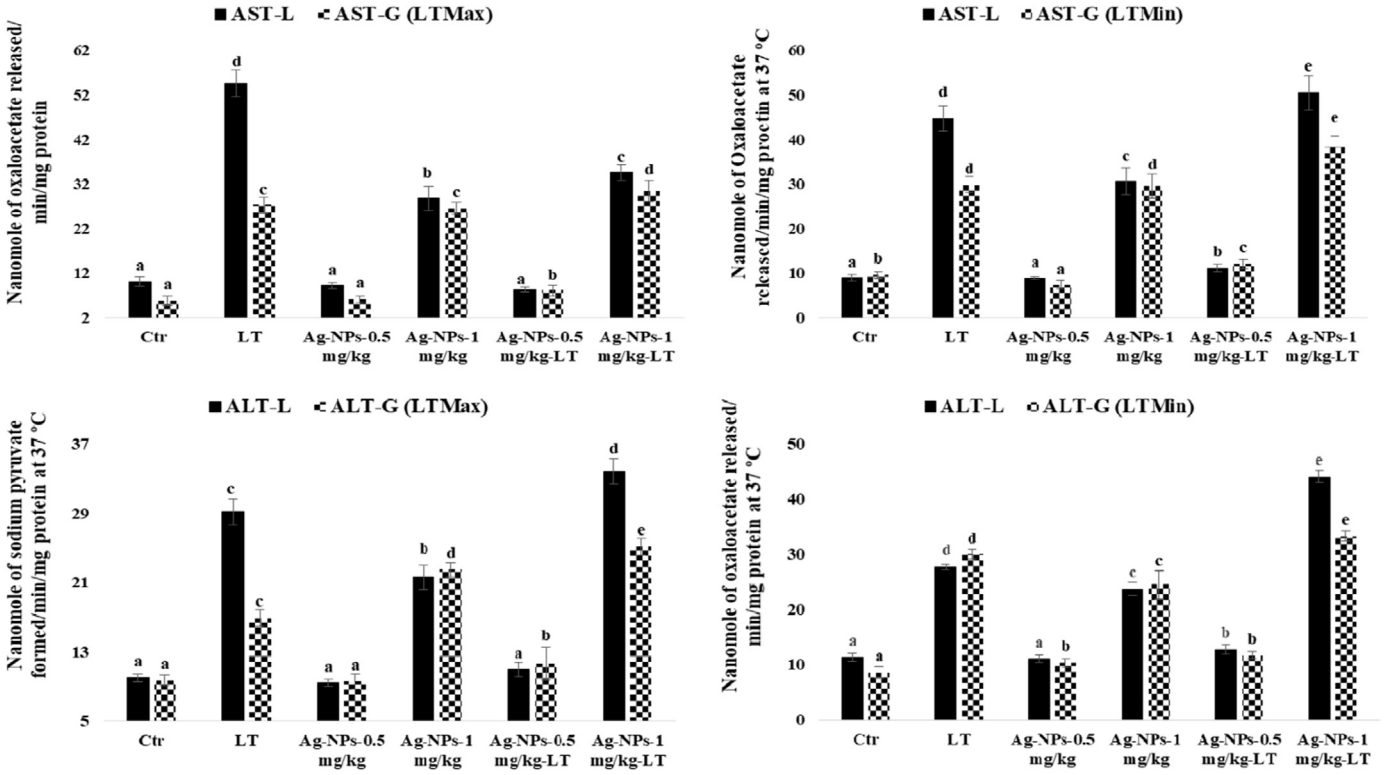

Fig. 6. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) on liver and gill after lethal temperature minima (LTMin) and lethal temperature maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d, e) indicate significant differences.

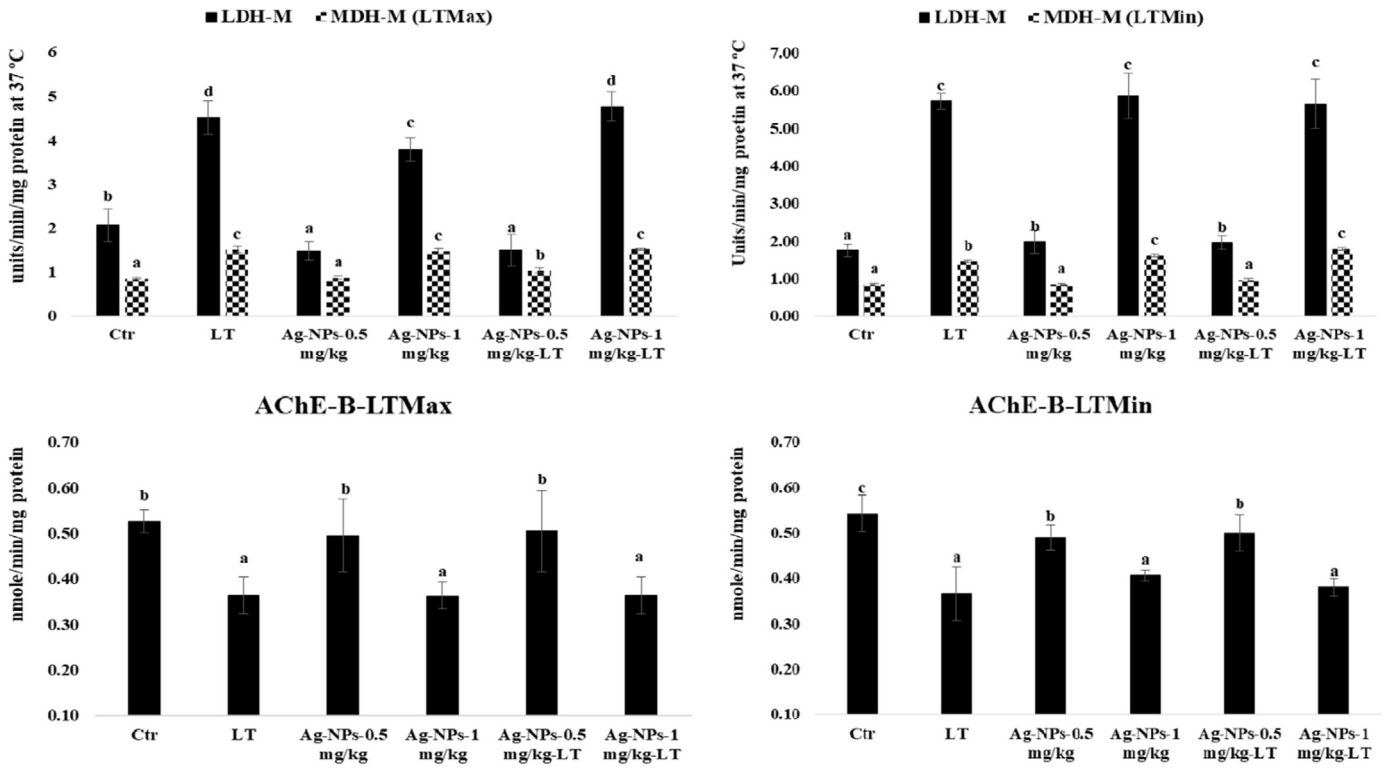

Fig. 7. Effect of different level of dietary silver-nanoparticles (Ag-NPs) on lactate dehydrogenase (LDH), malate dehydrogenase in muscle and neurotransmitter (AChE) enzyme in brain after lethal temperature minima (LTMin) and lethal temperature maxima (LTMax) of *C. striatus* reared under Pb and high temperature for 50 days. Values are expressed as mean ± SE, n = 3. Bars bearing different letters (a, b, c, d) indicate significant differences.

0.5 mg/kg diet with or without stressors (Pb and high temperature) significantly improved CTMin LTMin and CTMax LTMax, but supplementation of Ag-NPs @ 1 mg/kg diet did not improve thermal tolerance of *C. striatus*. This can be attributed to the toxic effect of Ag-NPs @ 1 mg/kg diet on fish, whereas Ag-NPs @ 0.5 mg/kg diet could efficiently enhance thermal expression, thermal regulatory mechanism and control expression of heat shock proteins which are responsible for repair and prevention of damage of cellular stress associated protein denaturation at lower or higher temperature (Nakano and Iwama, 2002). Earlier, researchers found that beneficial nutritional supplements help in remarkable enhancement of thermal tolerance in fish (Tejpal et al., 2014; Gupta et al., 2010; Kumar et al., 2014a; 2017c; 2016b). Our previous reports on selenium and zinc nanoparticles (Kumar et al., 2017a, 2017b) also supports our present finding of enhancement of thermal tolerance in fish. Ag-NPs are widely used as an antimicrobial agent, besides their property to improve the immunological status in fish. The earlier study also emphasized the role of silver as an effective antimicrobial agent against the infection (Dakal et al., 2016; Jung et al., 2008).

The thermal regulation in fish is directly controlled by the endocrine system in which hypothalamo-pituitary-adrenocortical is the key point (Joseph-Bravo et al., 2015a). The hypothalamic–pituitary–thyroid (HPT) axis has a major role in the regulation of metabolism and energy homeostasis (Joseph-Bravo et al., 2015a, 2015b) in addition to HPT, the thyroid hormone (TH) have a role in thermal response during cold stress (Little and Seebacher, 2014). The model based on forecasting the effect of climate change on species distribution called oxygen and capacity limited thermal tolerance (OCLTT) model (Portner et al., 2014; Woodin et al., 2013; Fry and Hart, 1948). Fry and Hart (1948), mentioned that cardiorespiratory system failure is the principal determinant of both upper critical temperature where animals lose equilibrium and the realized environmental thermal niche (Portner, 2010). The reason for this failure might be the requirement of oxygen to the cell such as mitochondria at high temperatures and increasing ATP demands of the tissues that surpass the oxygen supply capacity of the cardiovascular system. The OCLTT model suggested that the early evolutionary principle in animals has been modified according to life stage (Portner and Farrell, 2008) or climate zone (Portner, 2006; Beers and Sidell, 2011; Portner et al., 2013), or during the evolution of air-breathing fishes (Giomi et al., 2014). In the present study suggest that the use of Ag-NPs might be helpful in the controlling regulation of hypothalamo-pituitary-adrenocortical. However, the mechanistic study is required to understand the cause of thermal tolerance using Ag-NPs.

The thermal tolerance of fish decreases with contamination and pollution of metal and pesticide (Patra et al., 2007; Kumar et al., 2014a; 2016a; Kumar et al. 2018b) which drastically alter the metabolic enzymatic systems along with anti-oxidant defence system of the fish (Abele and Puntarulo, 2004; Portner, 2002b). Uncontrolled temperature triggers over production of reactive oxygen species (ROS), creating oxidative stress (Kumar et al., 2014a, 2016a) in the aquatic animal. The overproduction of free radicals leads to alterations in the membrane fluidity and phospholipid bilayer dynamics of the tissues. In polluted aquatic ecosystems, thermal stress or any kinds of destructive process can result into oxidative stress through overproduction of reactive oxygen species (ROS) which cannot be detoxified by the stressed aquatic organism (Halliwell, 1994; Ahmed, 2005; Madeira et al., 2013; Kumar et al. 2014b). ROS in aquatic systems are naturally synthesized by cell and play important role in the maintenance of cellular activity including inter and intracellular signaling (Halliwell and Gutteridge, 1999). It can cause extensive injury to the cell in the case of overproduction. In the present study, the oxidative stress in terms of CAT, SOD and GST were highly elevated with concurrent exposure to Pb and high temperature and fed with control diet and Ag-NPs @ 1 mg/kg diet extensively during LTMin and LTMax. The oxidative stress was drastically reduced with supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors, as indicated by the reduced oxidative stress (CAT, SOD and GST). It might be due to the fact that Ag-NPs have the capacity to enhance antioxidant defences system as it controls ROS level through

Table 2
Silver and lead bioaccumulation in muscle, liver, gill, kidney and experimental water during experimental period of 50 days.

Treatments	Silver (Ag), Tissues (mg/L), Water- µg/L					Lead (Pb), Tissues (mg/L), Water- µg/L				
	Muscle	Liver	Gill	Kidney	Water	Muscle	Liver	Gill	Kidney	Water
Control	0.02 ± 0.002	0.05 ± 0.001	0.01 ± 0.001	0.04 ± 0.001	0.14 ± 0.002	0.01 ± 0.001	0.02 ± 0.001	0.01 ± 0.002	0.04 ± 0.001	0.17 ± 0.001
LT	0.03 ± 0.001	0.05 ± 0.002	0.02 ± 0.001	0.04 ± 0.002	0.15 ± 0.003	0.42 ± 0.001	0.58 ± 0.01	0.45 ± 0.01	0.79 ± 0.04	420.69 ± 35.37
Ag-NPs-0.5 mg/kg	0.04 ± 0.002	0.11 ± 0.001	0.04 ± 0.002	0.17 ± 0.001	0.25 ± 0.001	0.04 ± 0.002	0.02 ± 0.001	0.03 ± 0.003	0.04 ± 0.001	0.37 ± 0.002
Ag-NPs-1 mg/kg	0.05 ± 0.002	0.16 ± 0.003	0.06 ± 0.002	0.18 ± 0.001	0.28 ± 0.002	0.06 ± 0.003	0.05 ± 0.002	0.05 ± 0.001	0.05 ± 0.001	0.45 ± 0.02
Ag-NPs-1 mg/kg-LT	0.07 ± 0.003	0.10 ± 0.002	0.05 ± 0.002	0.15 ± 0.001	0.24 ± 0.001	0.15 ± 0.001	0.59 ± 0.02	0.36 ± 0.03	0.86 ± 0.02	207.00 ± 18.40
Ag-NPs-1 mg/kg-LT	0.08 ± 0.001	0.18 ± 0.001	0.05 ± 0.003	0.19 ± 0.002	0.33 ± 0.002	0.45 ± 0.002	0.62 ± 0.01	0.33 ± 0.04	0.95 ± 0.01	602.23 ± 28.64

Values are expressed as mean ± SE, n = 3.

activation of CAT, SOD and GST levels as well as non-enzymatic defences such as amino acids, tocopherol and vitamins E, K and C (Martínez-Álvarez et al., 2005; Grim et al., 2010) through singlet oxygen at the production site, and reduce the flux of reduced oxygen intermediates (O_2 and H_2O_2) preventing the production of HO radicals, which are the most damaging oxygen species (Cadenas, 1989; Fridovich, 1998; Halliwell and Gutteridge, 1999). In our previously reported work (Kumar et al., 2014a; 2016a), it was found that the use of pyridoxine and lecithin successfully reduced the level of oxidative stress against stressors and enhanced thermal tolerance of the fish. However, no such finding has been reported with Ag-NPs.

AST and ALT are the strong bioindicators for signaling stress in fish. In the present study, these bioindicators were highly affected by concurrent exposure to Pb and high temperature and fed with control and Ag-NPs @ 1 mg/kg diet during LTMin and LTMax, indicating that fishes in these two groups were under stress. Elevated levels of AST and ALT are mainly due to tissue damage (Manna et al., 1996) as reflected in our study, as the liver histopathology showed several marked signs of damage due to concurrent exposure to Pb and high temperature and fed with control and Ag-NPs @ 1 mg/kg diet (Kumar et al., 2018c). The alternation in protein metabolic enzymes also might be due to increased proteolysis, enhanced protein catabolism, cellular alteration in the liver and gill as reflected in anti-oxidative status and other enzymes. The use of Ag-NPs @ 0.5 mg/kg diet has the capacity to reduce AST and ALT level during LTMax and LTMin, probably due to the role of Ag-NPs in controlling the pathway of protein metabolic synthesis. Our previous study reported the use of dietary Se-NPs for improvement of protein metabolic enzymes (Kumar et al., 2017a).

LDH and MDH are the key enzymes for the glycolytic cycle and directly sense the stress level in different tissues in fish. In our present study, the level of LDH and MDH were highly elevated with concurrent exposure to Pb and high temperature and fed with control and Ag-NPs @ 1 mg/kg diet after LTMin and LTMax, which might be due to process of anaerobic glycolytic pathway leading to the conversion of lactate and malate to glucose which acts as major source of energy during stress (Rajamanickam and Muthuswamy, 2008). LDH has an important role in the glycolytic cycle of forming pyruvic acid in anaerobic condition during metabolism. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply. Reduced LDH and MDH activities were observed in supplementation of Ag-NPs @ 0.5 mg/kg diet with or without stressors group after LTMin and LTMax.

Neurotransmitter enzyme such as acetylcholine esterase (AChE) activities were remarkably inhibited in the group exposed to Pb and high temperature and fed with control and Ag-NPs @ 1 mg/kg diet after LTMin and LTMax. AChE inhibition might be due to the production of the toxins by multiple stresses of Pb and high temperature, since, neurotransmitters work for afferent nerve fibers (Kumar et al., 2017a). Further, AChE has been used as a biomarker for pollution/contamination in the aquatic ecosystem. The toxic nature of Pb provokes binding of Pb to functional groups of protein like imidazole, sulfhydryl and carboxyl (Najimi et al., 1997). The toxicity of Ag-NPs also depends upon the concentration used for experimental feeding. Nevertheless Ag-NPs @ 0.5 mg/kg diet with or without stressors (Pb and high temperature) helps to retain inhibited AChE activities up to more than 50%, so it indicates that dietary Ag-NPs acts based on dose response. The underlying mechanism of improvement in acetylcholine esterase activities due to dietary Ag-NPs unknown to us. Therefore, further study might provide greater insight into understanding the molecular mechanism of improvement.

5. Conclusion

There is a significant impact of global climate change effects are reflected in the aquatic ecosystem as unpredicted temperature (high

and low) fluctuations, adversely affecting the production of aquatic animals such as fish. Therefore, it is absolutely imperative to sought an environmentally viable solution for minimizing such adverse impact on the fish for sustainable aquaculture. The present study shows that dietary silver nanoparticles (Ag-NPs) play an important role in the enhancement of thermal tolerance, improve the anti-oxidative status and maintain others biochemical enzymes in *C. striatus*. Therefore, it is recommended that Ag-NPs could be used @ 0.5 mg/kg to overcome the impact of thermal and pollutant-induced stress in fish.

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Conflict of interest

All authors declare that they have no conflict of interest.

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